DNA repair: ***RAD* alert**

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Mammalian homologues of two important yeast genes involved in DNA double-strand break repair and recombination, *RAD51* and *RAD54*, have been isolated. Knock-out mutations of the genes in mice reveal both reassuring similarities to, and surprising differences from, the analogous mutant phenotypes in yeast.

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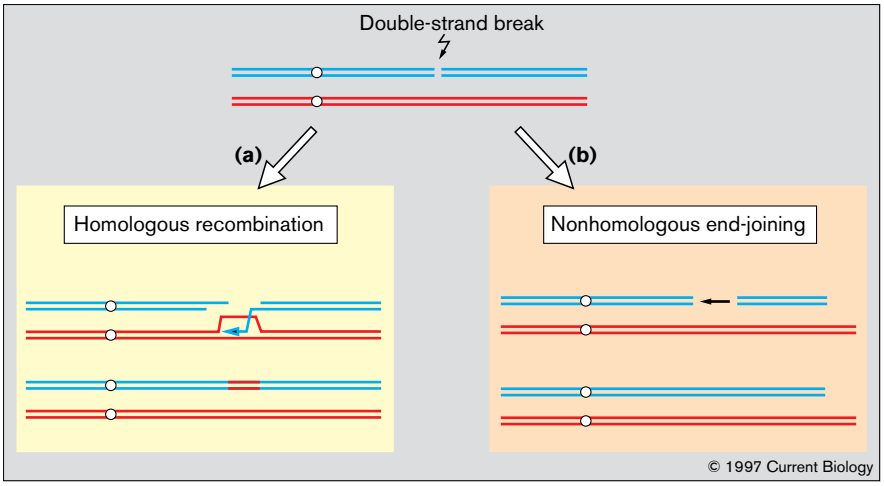
The existence of homologous recombination in mammalian cells was demonstrated more than ten years ago, but our understanding of the enzymology of the process remains sketchy at best and until very recently no mutant cell lines deficient in homologous recombination had been identified. Even worse, it turned out to be difficult to assign to homologous recombination a meaningful *in vivo* function, at least in mitotic cells. This is now changing with the isolation and characterization of mammalian homologues of budding yeast's *RAD* genes, which are required for genetic recombination and have well-defined physiological functions in this single-celled eukaryote.

The most significant role for genetic recombination in mitotic cells is to repair DNA damage, mainly the DNA double-strand breaks that can arise from the collapse of replication forks, ionizing radiation, the action of endonucleases and topoisomerases, or mechanical stress during chromosome segregation. In lower eukaryotes, such as the budding yeast *Saccharomyces cerevisiae*, double-strand breaks are repaired almost exclusively by the homologous recombination machinery (Figure 1a) [1]. When homologous repair is prevented, only about one in a hundred cells can repair double-strand breaks by illegitimate, or nonhomologous, end-joining. Yeast cells carrying *RAD* gene mutations that prevent homologous recombination, such as *rad51*, *rad52* and *rad54*, are thus profoundly sensitive to ionizing radiation, whereas cells carrying mutations in genes required for illegitimate end-joining — such as yeast homologues of *Ku70* and *Ku86* — show a normal ability to repair radiation-damaged DNA.

In mammalian cells, by contrast, nonhomologous end-joining appears to be the predominant pathway of double-strand break repair (Figure 1b). For example, when transfected DNA integrates into a host chromosome — a process that involves double-strand break repair — nonhomologous end-joining usually outperforms homologous recombination by at least 100:1. This may in part reflect the greater problem exogenous DNA faces in finding an appropriate homologous sequence in a mammalian genome, where the ratio of nonhomologous to homologous targets is much greater than in yeast. However, the apparent advantage of nonhomologous repair pathways may also depend on the nature of the DNA substrates. Recent studies of recombination in which a site-specific double-strand break

Figure 1



Double-strand DNA breaks can arise in a number of circumstances, such as in response to ionizing radiation. **(a)** In lower eukaryotes, such as yeast, double-strand breaks are repaired almost exclusively by homologous recombination. **(b)** In mammalian cells, however, double-strand breaks are repaired predominantly by nonhomologous end-joining.

was introduced into a chromosome suggest that homologous recombination might in fact be an efficient pathway of double-strand break repair in mammalian cells.

Sargent *et al.* [2] found that a site-specific double-strand break, created by expressing the yeast intron-encoded endonuclease I-*Sce*I in mammalian CHO cells, stimulated homologous recombination about 100-fold, whereas nonhomologous end-joining was stimulated about 1,000-fold. Although nonhomologous recombination still prevails, the fraction of double-strand breaks repaired by homologous recombination seems to be much higher than in the case of transfected DNA. Moreover, the efficiency of homologous events might be greatly underestimated, as homologous repair using an intact sister chromatid could not have been scored in the assay used. These results suggest that chromatin-associated chromosomal DNA is a better substrate for homologous recombination than naked exogenous DNA. The idea that the pathway chosen for DNA repair depends on the packaging state of the DNA is not unreasonable, in view of studies in yeast showing that the requirement for the DNA repair and recombination genes *RAD51* and *RAD54* is also DNA-structure-dependent [3].

Recently, vertebrate homologues of both *RAD51* and *RAD54* yeast genes have been cloned and 'knock-out' mutations of these genes have been created in mice to assess their phenotypes. The Rad51 protein shows strong sequence homology to bacterial RecA, which promotes the pairing of homologous DNA molecules and strand-exchange reactions that initiate general genetic recombination. Both yeast Rad51 and its human homologue, hRad51, can catalyse strand-exchange reactions *in vitro* that are similar, but not identical, to those catalysed by RecA [4,5]. However, there is growing evidence that the way Rad51 and its homologues act may be fundamentally different from the action of RecA. Unlike RecA, which appears to act largely alone, yeast Rad51p may function as part of a 'recombinosome', as Rad51 has been shown to interact with Rad54, as well as with Rad52 and other Rad proteins. A similar interaction between human Rad51 and Rad52 proteins has been reported [6].

The role of Rad54 remains elusive. It is a member of the Swi2/Snf2 family of helicase-like proteins that may play a role in chromatin remodeling. In budding yeast, *rad51* and *rad54* null mutants have very similar mitotic phenotypes: the cells are viable but show a high sensitivity to ionizing radiation and the radiomimetic drug methyl methane sulfonate, and reduced levels of spontaneous recombination. Although *rad51* and *rad54* mutants are deficient in double-strand-break-induced gene conversion between homologous chromosomes, they can still carry out DNA strand invasion leading to an alternative DNA repair pathway, break-induced replication [7]. Operation of this alternative repair pathway leads to a loss of heterozygosity of marker alleles distal to the double-strand break.

The meiotic phenotypes of *rad51* and *rad54* mutants are quite different, however, as the *rad51* mutation markedly reduces recombination, whereas *rad54* has little effect on recombination. This difference may be difficult to analyze, in view of the fact that there is an as yet uncharacterized homologue of *RAD54*, *RDH54*, that is important in both mitotic and meiotic cells, as well as a meiosis-specific homologue of *RAD51* called *DMC1*. Recently, homologues of both *RAD51* and *RAD54* have been identified in another yeast, the fission yeast *Schizosaccharomyces pombe*. The phenotypes caused by null mutations of these two genes are very similar to those seen in budding yeast [8].

The *RAD54* function appears to be at least partially conserved during evolution; for example, the human homologue of *RAD54* (*hHR54*) will complement the methyl methane sulfonate sensitivity of a yeast *rad54* mutant [9]. Two recent papers [10,11] have now reported that vertebrate cell lines lacking *RAD54* homologues display strikingly similar phenotypes to their yeast equivalents. In a mouse embryonic stem (ES) cell line carrying knock-out mutations of both *mRAD54* alleles, the efficiency of homologous recombination with transfected DNA was reduced five-to-ten-fold in comparison with a control $mRAD54^{+/-}$ cell line [10]. Furthermore, the $mRAD54^{-/-}$ cells were also two-to-three times more sensitive than wild-type cells to γ-irradiation and methyl methane sulfonate.

These results provide strong genetic evidence that *mRAD54* is involved in homologous recombination in mice and that homologous recombination contributes to double-strand-break repair; however, the γ-ray sensitivity of *RAD54*-deficient cells seems to be less pronounced than that of cells with mutations in *Ku86*, a participant in the nonhomologous end-joining pathway. Very similar data were reported for the chicken-derived cell line DT40 [11]. Cells homozygous for a knock-out mutations of the chicken *RAD54* homologue exhibited a reduced rate of immunoglobulin gene conversion, a lower efficiency of gene targeting and increased sensitivity to γ-rays and methyl methane sulfonate [11].

In contrast to the results on *RAD54* homologues, studies of mammalian *RAD51* homologues have provided some real surprises. It has proven impossible to establish mouse ES cell lines homozygous for knock-out mutations of *mRAD51*, and mice with a $mRAD51^{-/-}$ genotype die early in embryonic development [12,13]. The inviability of *RAD51*-deficient mice is unexpected, given that most chromosomal double-strand break repair seems to involve nonhomologous repair pathways and that animals lacking the key component of this pathway, the Ku86 protein, are alive and more-or-less well, albeit smaller, radiation-sensitive and

immune deficient [14]. Another recent study [15] has shown that the absence of Ku86 in CHO cells markedly reduces nonhomologous end-joining, without affecting homologous recombination, a phenotype very similar to that of budding yeast mutant for the *Ku86* homologue [16].

The lethality of *RAD51*-deficient mammalian cells implies that this gene is involved in a process (or processes) essential for cell viability — processes that apparently do not require *RAD54*, as this gene is not essential for growth. One approach to the problem of identifying essential *RAD51*-dependent processes would be to look for interactions of RAD51 with other proteins, and numerous and diverse interactions have indeed been found. As well as interacting with hRad52 [6], hRad51 interacts with a human homologue of the yeast ubiquitin-conjugating enzyme Ubc9 and with the ubiquitin-like protein Ubl1 [6]. Human Rad51 protein was also found to be associated with a large RNA polymerase II complex, which also contained DNA-dependent protein kinase (a component of the nonhomologous end-joining machinery), DNA polymerase ε and some proteins involved in excision repair [17]. At this point, one can imagine hRAD51 influencing nonhomologous and homologous double-strand break repair, transcription, protein turnover and replication! Not all of these interactions may prove to be significant *in vivo*, of course.

Most strikingly, *RAD51* appears to be intimately involved in cancer biology. Human Rad51 protein interacts physically with the tumor suppressor protein p53 [18]. In mice, embryos deficient for both *mRAD51* and *p53* develop further than embryos deficient for *mRAD51* alone [13]. Human and mouse Rad51 also interact with two breast cancer tumor suppressor gene products, Brca1 [19] and Brca2 [20], although it was not shown whether the interaction is direct or mediated through other proteins. Furthermore, *mBRCA2*$^{-/-}$ mice die early during development, with a phenotype very similar to that of *mRAD51*$^{-/-}$ mice [20]. This cancer connection suggests that the reason *RAD51* is required for the viability of mouse cells and embryos is because Rad51 is involved in cell growth control, and not just in the repair of DNA damage that arises during replication and chromosome segregation; the loss of other genes that might have been expected to cause similarly severe defects in DNA repair, such as *RAD54* or *KU86*, has far fewer pleiotropic effects.

The lethality of knocking-out *RAD51* in mice contrasts with the viability of *rad51* yeast mutants. However, the idea that *RAD* genes are involved in essential processes is not without precedent, even in yeast: deletion of yeast *RAD54* proves to be lethal in conjunction with deletion of *SRS2*, which encodes a helicase implicated in post-replication DNA repair. Furthermore, transcription of *RAD51* is cell-cycle-regulated, peaking in late G1 to early S phase, concurrent with DNA replication enzymes [21]. This raises the possibility that, in addition to its role in DNA repair, *RAD51* has a non-essential function during DNA replication. Is this an indication that the essential function of mammalian *RAD51* genes might have been 'conceived' early in evolution?

Much remains to be learned about the *RAD* genes of mammals. Are there additional homologues of *RAD51* or *RAD54*? What will be the phenotypic effects of knocking-out mammalian homologues of *RAD52* or other *RAD* genes? Mammalian homologues of most of these genes have already been identified and the effects of inactivating them in mice should soon be known. Finally, would double mutations in mouse cells that remove both homologous (*rad54*) and nonhomologous (*ku86)* recombination reveal hypersensitivity to ionizing radiation? It will be fascinating to see the degree to which DNA repair functions have been conserved and the new ways that the *RAD* gene products have been put to work.

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